

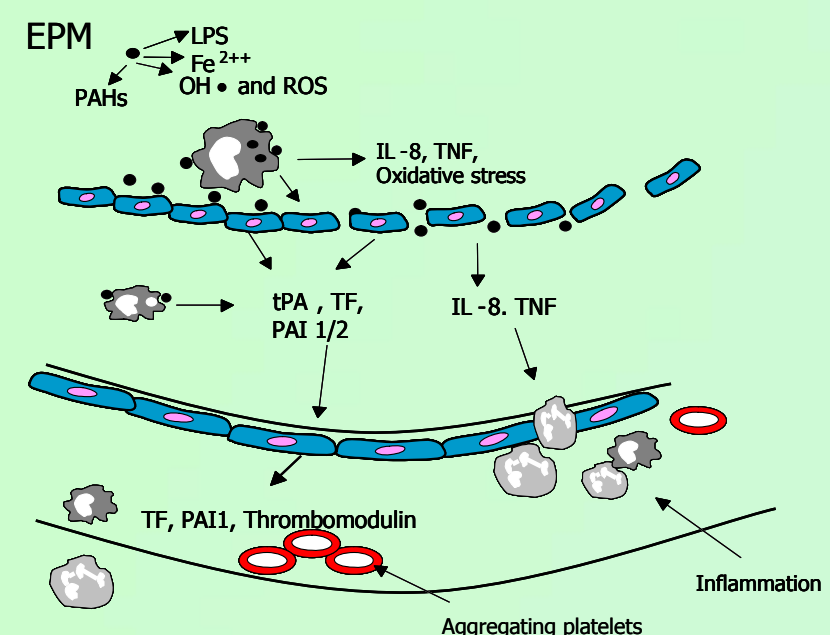
# Mechanisms of Cardiovascular Health Effects Associated with Environmental Particles

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## Environmental Issue

- Pollution particles have been associated with effects on blood pressure and blood coagulation factors in epidemiological studies.
- In experimental studies, differences in heart rate variability following particle exposure have been identified, however the mechanisms behind blood coagulation and systemic effects caused by PM-mediated pulmonary inflammation remain unclear.
- Metal content of particles may explain toxicity and toxicity differences between particle sources.
- Studies using Ottawa and Utah Valley particles have identified zinc as an important mediator of toxicity in particle animal exposures.



## Hypothesis

Pulmonary exposure to zinc containing Emission Particulate Matter (EPM) and zinc causes pulmonary and cardiac inflammation/injury, causes lung and heart thromboses, and alters the coagulation and fibrinolysis.

These effects are more readily apparent in cardiovascular compromised spontaneously hypertensive (SHR) than Wistar Kyoto (WKY) normotensive rats.

## Methods

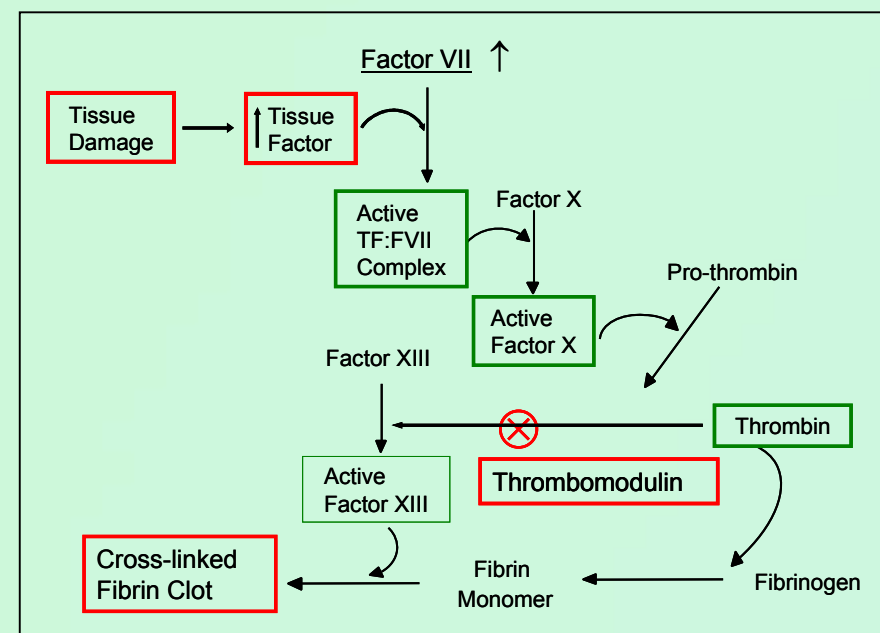
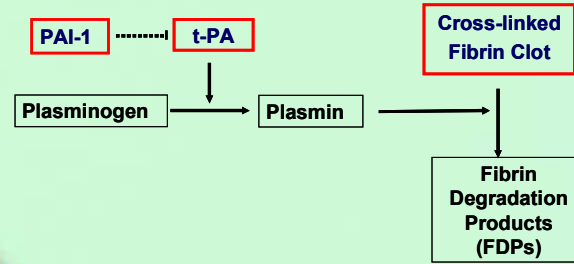
- Wistar-Kyoto (WKY - normotensive) and Spontaneously hypertensive (SHR) rats were exposed by nose only inhalation (10 mg/m<sup>3</sup>, 6 h/day, 1 d/week for 16 weeks), or instilled with 3.3 mg/kg oil-derived combustion particle (EPM) from two sources. These particles were analyzed for their metal composition.
- Sprague Dawley (SD), WKY and SHR rats were instilled with 1 or 2 µmol/kg Zinc sulphate.
- Lung inflammation and blood parameters and lung and cardiac tissue gene expression (real time and RT-PCR) were determined 1 and 24 hours post instillation.
- Lung and cardiac injury was evaluated 1, 4, 24, 48 and 96 hours post instillation in WKY rats.
- Myocardial lesions were characterized by immunohistochemical staining for troponin and apoptosis in cardiac sections.

## Extrinsic clotting cascade

- Tissue injury and inflammation cause Tissue Factor (TF) activation which upon combination with FVII forms an active TF:FVII complex and therefore initiates the coagulation cascade.
- TF expression can be induced in a number of cell types in response to damage or inflammatory mediators.
- We believe that PM-induced inflammation may increase Tissue factor expression in the lung and therefore enhance coagulation.

## Fibrinolysis – resolution of thrombi

- Cross linked fibrin clots are degraded by the activation of plasminogen to plasmin which causes the degradation of thrombi.
- This activation is initiated by tissue plasminogen activator (tPA).
- tPA is in turn inhibited by plasminogen activator inhibitor (PAI)-1.
- A decrease in tPA or an increase in PAI-1 alters the fibrinolytic balance to inhibit clot resolution in the lung or associated with atherosclerotic plaques.



## Particle Composition

### EPM-1 composition

Component	Water-leachable (µg/mg)	1M HCl-Leachable (µg/mg)
Sulfate	107.0	134.9
Zinc	14.5	14.6
Nickel	3.0	3.2
Iron	2.5	65.4
Vanadium	0.1	15.4
Cobalt	0.1	0.1
Copper	0.1	0.2
Manganese	0.4	0.5

### Composition of EPM-2

Component	Water-leachable (µg/mg)	1M HCl-Leachable (µg/mg)
Sulfate	212.68	221.57
Zinc	11.47	15.39
Nickel	6.94	14.77
Vanadium	1.25	32.88
Copper	0.23	1.13
Lead	0.042	1.70
Iron	0.016	15.45
Potassium	17.18	19.52
Magnesium	12.80	15.12

### Zinc levels of select ambient particles:

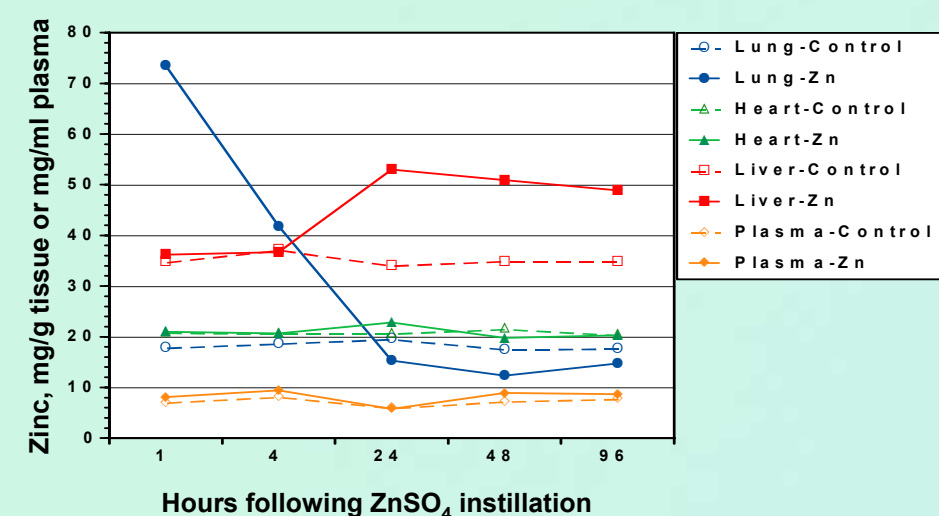
Location	PM-type	Zn Conc. (µg/mg)	Reference
Boston, MA, Power Plant	Combustion	14.5	Kodavanti et al, 2002
Ottawa, Canada	Ambient, bag house	10.4	Adamson et al, 2000
Utah Valley, UT	Ambient filter extract	0.074	Dye et al, 2001
NIST*, St Louis, MO	Ambient	3.5	Costa and Dreher, 1997
Lodz, Poland	Ambient	11.9	Cassee et al (unpublished)
Baltimore, MD	Ambient	1.1	Walters et al, 2000
Lahore, India	Ambient	27.7	Harrison and Yin, 2000
Birmingham, UK	Ambient	0.30	Harrison and Yin, 2000

### Florida Power Plant Fly Ash composition

Component	Water-leachable (µg/mg)
Zinc	6.55
Iron	130.4
Vanadium	272.8
Nickel	218.9
Copper	1.5

EPM-1 and 2 have relatively sizable amounts of zinc, and relatively (for EPM) low levels of Vanadium, Iron, Nickel and Copper.

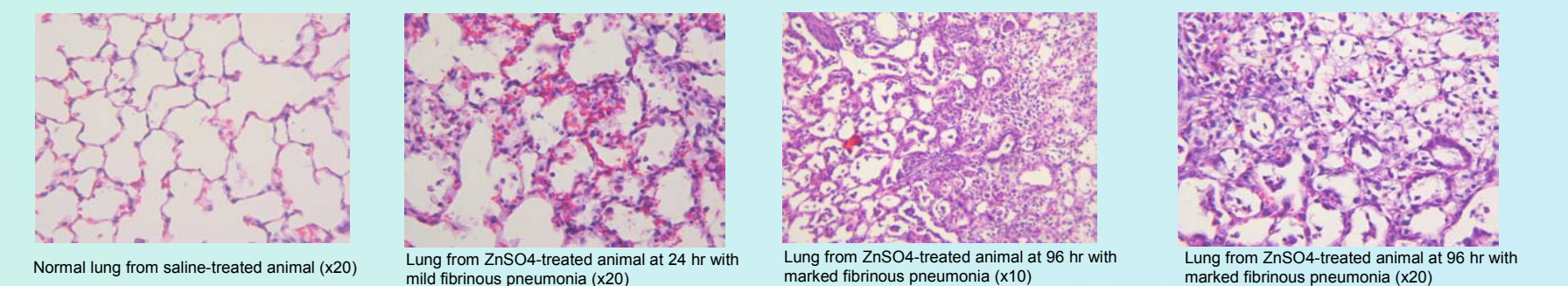
## Kinetics of zinc distribution in the body following intratracheal instillation of ZnSO<sub>4</sub> (2 µmol/kg) in WKY rats



•Zinc concentration decreases rapidly in Lung tissue to below control levels at 24 hours post instillation.

•Liver Zinc concentration increases 4 hours following instillation and remains raised above control levels beyond 96 hr post instillation.

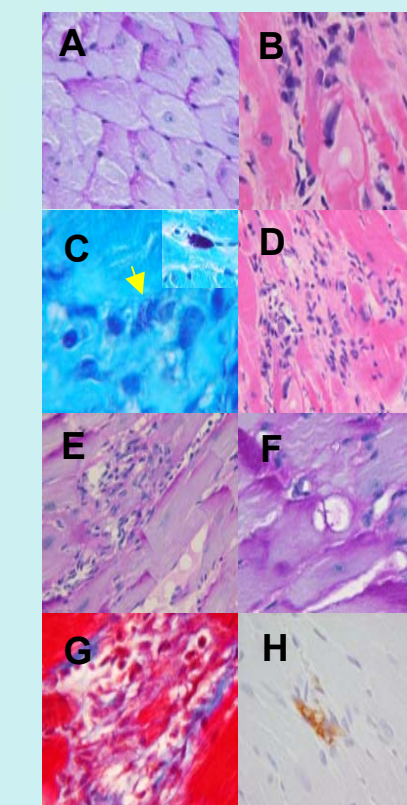
## Inflammation caused by Zinc exposure in the lungs of WKY rats



Intratracheal Zinc causes an acute inflammatory reaction in the lungs of exposed rats which extends beyond 96 hours post exposure.

## EPM-1 and Zinc-mediated Cardiac injury

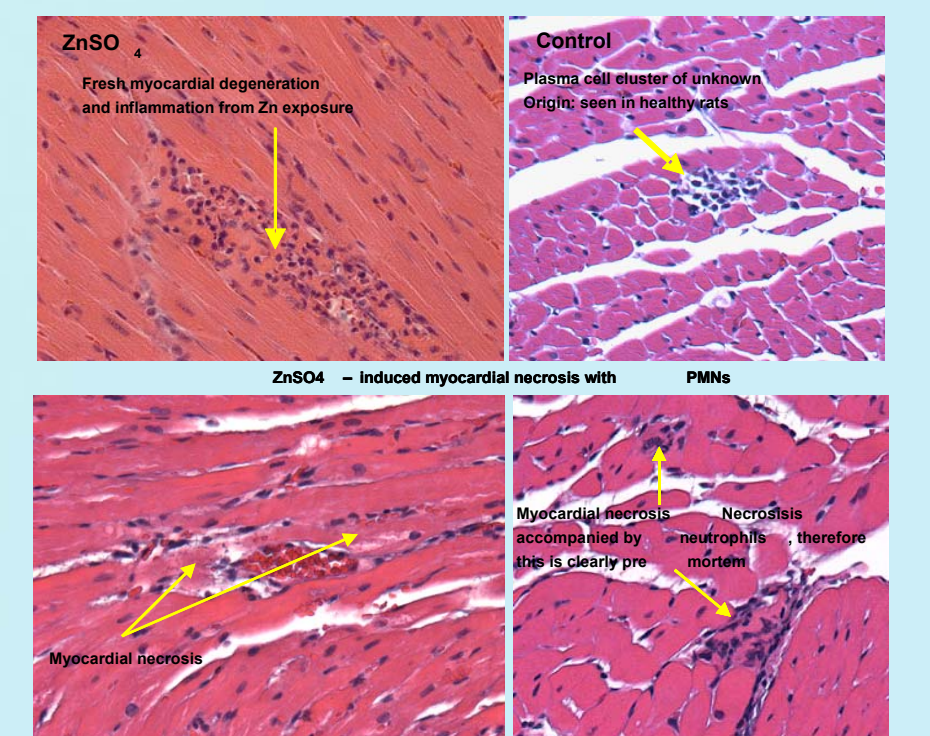
Myocardial injury in EPM-1 exposed WKY rats: long-term inhalation exposure



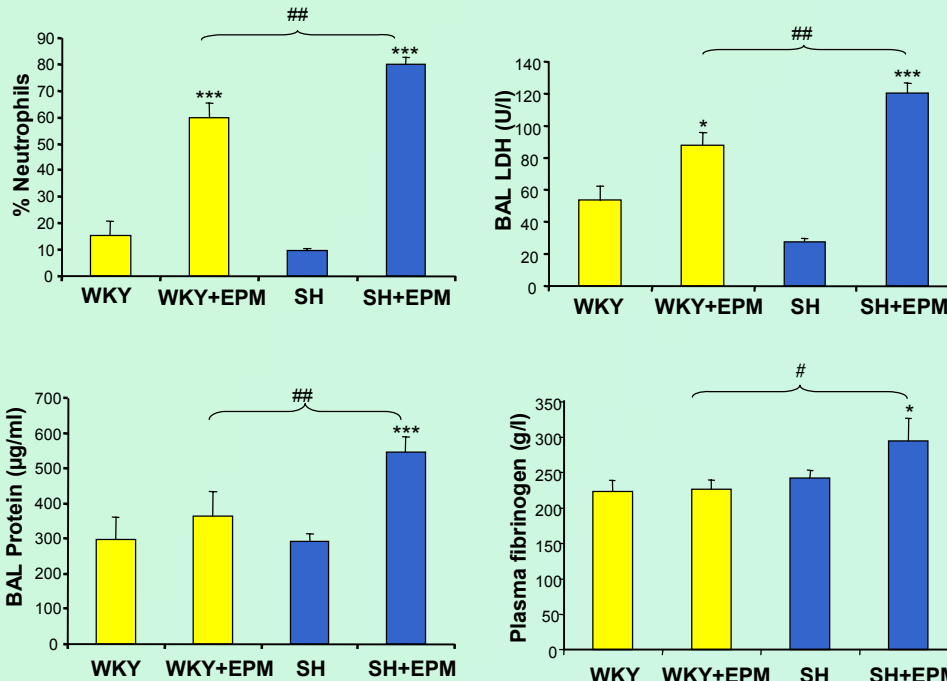
A=normal myocardium  
B=myocardial degeneration and inflammation  
C=mast cell degranulation  
D=myocardial degeneration and inflammation  
E=myocyte degeneration  
F=myocardial vacuolation  
G=myocardial fibrosis  
H=apoptosis

Acute EPM and Zinc pulmonary exposure caused injury and inflammation to the heart myocardium.

Myocardial injury in ZnSO<sub>4</sub>-exposed WKY rats: Acute intratracheal exposure (H&E)



## EPM-2 Pulmonary Injury



EPM-2-mediated lung injury (SH and WKY exposures)

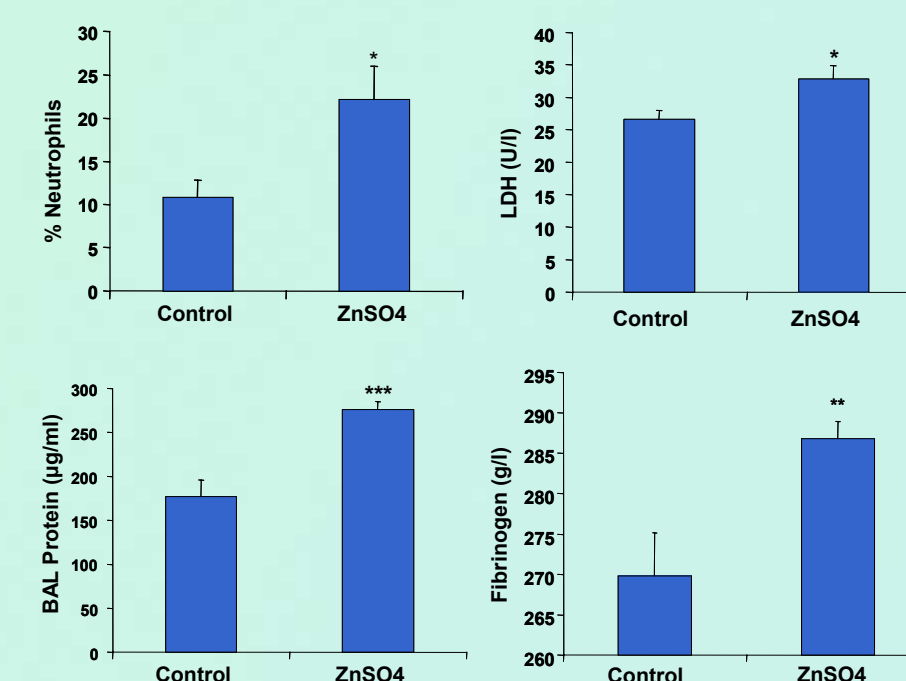
•For EPM dose response relationship see poster by Wichers *et al*.

•EPM significantly increased BAL neutrophils, protein and LDH.

•A systemic effect was present in SH rats as plasma fibrinogen was significantly increased following EPM exposure.

•All parameters were significantly increased in SH rats exposed.

## Zinc Pulmonary Injury



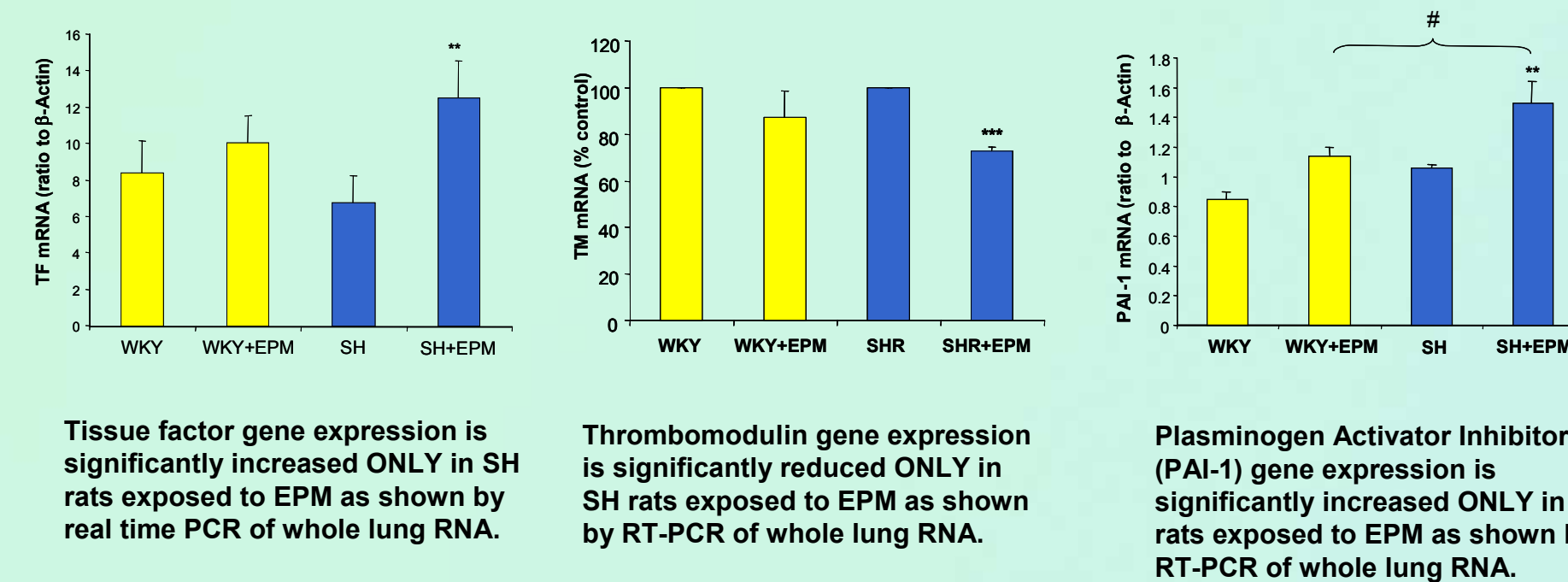
Zinc-mediated lung injury (SC rat exposures)

•Zinc significantly increased BAL neutrophils, protein and LDH compared to control saline exposures.

•Plasma Fibrinogen was significantly increased in zinc exposures compared to saline control exposures indicating a systemic effect of pulmonary zinc exposure.

## Thrombotic and cardiovascular effects of acute EPM-2 and Zinc exposure

### EPM-2 causes pro-thrombotic gene expression changes

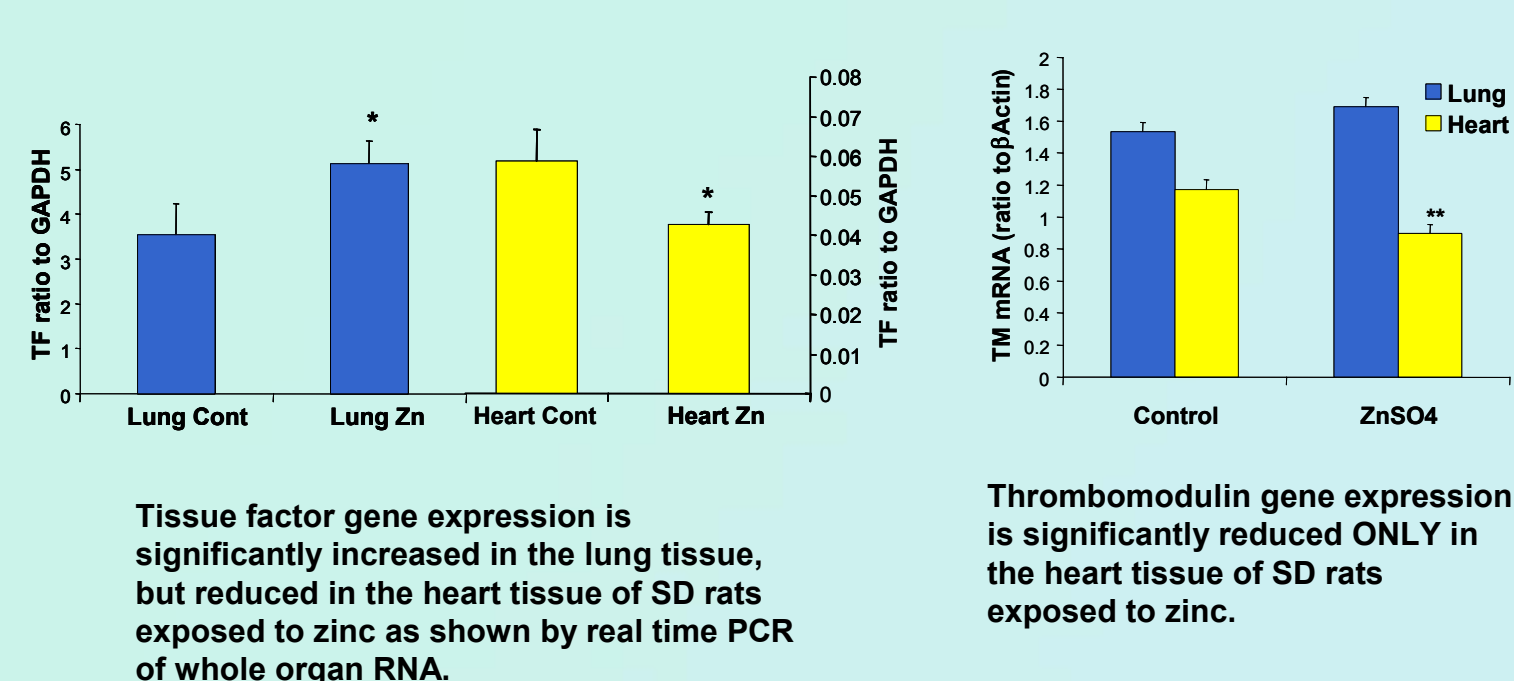


Tissue factor gene expression is significantly increased ONLY in SH rats exposed to EPM as shown by real time PCR of whole lung RNA.

Thrombomodulin gene expression is significantly reduced ONLY in SH rats exposed to EPM as shown by RT-PCR of whole lung RNA.

Plasminogen Activator Inhibitor-1 (PAI-1) gene expression is significantly increased ONLY in SH rats exposed to EPM as shown by RT-PCR of whole lung RNA.

### Zinc causes pro-thrombotic gene expression changes in heart and lung



Tissue factor gene expression is significantly increased in the lung tissue, but reduced in the heart tissue of SD rats exposed to zinc as shown by real time PCR of whole organ RNA.

Thrombomodulin gene expression is significantly reduced ONLY in the heart tissue of SD rats exposed to zinc.

## Conclusions

- We show that EPM and the major EPM metal component zinc cause cardiac and pulmonary inflammation and induce pro-coagulative gene expression which may be a mechanism for the formation of lung and myocardial microvascular thrombi. Zinc is one of the major transition metal-components of ambient PM.

- These results suggest that a pro-coagulative response to particle and zinc in the lung and heart may contribute to particle-mediated cardiovascular health effects in compromised populations.

- Oxidative stress is known to induce blood coagulation and tissue pro-coagulative effects and may be the mechanism by which environmental particles and zinc induce coagulation and health effects.

## Future Directions

- Since significant attention is given to particle translocation to the heart, especially ultrafines, our follow-up studies will investigate the contribution of soluble and non-soluble components in inducing endothelial cell changes in the lung microvasculature and cardiac coronaries.
- The role of oxidative stress and microvasculature thrombosis will be investigated using intervention strategies involving systemic use of antioxidants, such as Nacystelyn, and anti-thrombogenic factors, such as anti-Factor IXa for SH rats.

## Impact

These studies support the NAS recommended PM research need on identifying mechanisms and susceptibility with primary focus on how pulmonary exposure can lead to myocardial injury and cardiac mortality.